

AN EVALUATION OF POTENTIAL CRITERIA
FOR ESTIMATING AGE OF COYOTES

By

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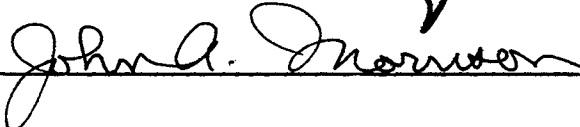
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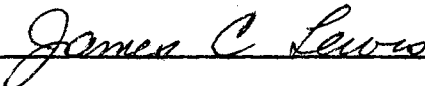
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CHAPTER I

INTRODUCTION

Interest in the dynamics of mammalian carnivore populations has resulted in the development of numerous aging methods. Currently, subjective criteria (tooth wear, closure of epiphyses and skull sutures, and other morphological characters) are used infrequently because of the low precision and accuracy inherent in such criteria. At present, attention is being given to concentric rings seen in histological preparations of dental cementum of certain mammals. Evaluation of these rings in a variety of known-age carnivores (Linhart and Knowlton 1967, Monson et al. 1973, Stoneberg and Jonkel 1966), as well as in various known-age herbivores (Gilbert 1966, Lockard 1972, McCutchen 1969, Ransom 1966, Thomas and Bandy 1973), strongly suggests that these rings are annual in nature. Klevezal and Kleinenberg (1967) have presented a review of other mammals that appear to have annual layers in dental cementum. Deposition of the first ring apparently occurs at various times among species; however, subsequent deposition probably occurs at the rate of one annulus per year.

Histologic preparations are expensive, time consuming and require expertise and equipment. Unless current histologic techniques for analyzing cementum annuli can be substantially simplified, there is a need for an accurate method of determining age that may be quickly and inexpensively applied to large samples.

In this study, three potential criteria of age that may satisfy the need for simplicity are examined for the coyote (Canis latrans). Eye lens weight, weight of the baculum and thermal contraction of tail tendons are evaluated in comparison to counts of cementum annuli in the canine teeth.

CHAPTER II

MATERIALS AND METHODS

Coyotes were collected from January 1972 through May 1973 throughout Oklahoma by steel trapping, using "coyote-getters" (cyanide guns), and "calling." Most trapped animals, and all of the coyotes collected using "coyote'getters." were obtained from McAlester Naval Ammunition Depot, McAlester, Oklahoma during a predator-control program. The majority of coyotes donated by hunters were taken by predator "calling" throughout the state. All persons actively collecting coyotes for the study were asked to freeze the animals as quickly as possible. Field conditions, at times, prevented immediate freezing; however, it is believed that all animals were frozen within 4 hr post mortem.

To provide known-age coyotes, it was assumed that the number of cementum annuli present was an accurate indicator of age, in years, of coyotes. It was further assumed that the first cementum annulus is laid down during the 23rd month of life and that the canine root canal is open until 8 months but closed at 9 months of age. These are reasonable assumptions in view of the work of Linhart and Knowlton (1967) who examined 30 known-age coyotes.

Capture dates for this study were known; therefore, it was possible to place study animals into a single-month age class if birth dates were known. Because most coyotes are born in April or May (Dunbar 1973, Gier 1968, Linhart and Knowlton 1967), a May birth date

was assumed. A possible difficulty arises with "young-of-the year" and "yearling" coyotes captured during December, January and February which do not show an annulus. These animals are either 8, 9, 10, or 20, 21, 22 months of age. Because Linhart and Knowlton (1967) observed that by 1 yr of age the canine pulp cavity has typically narrowed, animals in this study captured in December, January and February were assigned ages according to the relative width of the pulp cavity. For example, a coyote captured during December showing no annuli but having a wide pulp cavity was assigned an age of 8 months; a coyote captured in December showing no annuli but having a narrow pulp cavity was assigned an age of 20 months.

Lower canines were extracted from jawbones after heating them for 45 min at 15 lb. pressure in a commercial pressure cooker as suggested by Sergeant and Pimlott (1959). To determine whether pressure cooking might alter stainability of cementum structures, five jawbones were sawed in half and the canines from one group extracted by pressure cooking while the remaining jawbone halves were soaked in water at room temperature to facilitate extraction. Microscopic comparison of the prepared sections showed no appreciable difference. In addition, Crowe (1972) found that simple boiling does not adversely affect the stainability of bobcat (Lynx rufus) cementum annuli.

After extraction, teeth were stored in 70 percent methanol or decalcified in a 10 percent solution of HCl (37 percent concentration) at room temperature. Decalcification was complete in 48 hr, and difference between sections decalcified with HCl and those decalcified with formic acid, as recommended by Linhart and Knowlton (1967), was not evident in this study. However, decalcification in formic acid

solutions took in excess of 8 days and usually left the interior of canines only partially decalcified. Because complete decalcification is required for manual sectioning, a decalcifying solution of 10 percent HCl was used. Transverse serial sections of 80 to 150 micra thickness were cut manually from decalcified canines with a razor blade. Sectioning in this manner is not difficult if a fresh blade is used for each tooth. Serial transverse sections were selected over sagittal sections because: 1) sagittal sections were extremely difficult to take by hand, 2) the numerous transverse serial sections increased the probability of viewing the area of optimum definition of annuli, and 3) annuli in transverse sections could be traced about the entire circumference of the section.

Sections were processed and stained using a modification of the method described by Erickson and Seliger (1969). Sections were processed through the following baths: de-ionized water for 1 min, a saturated solution of lithium carbonate in 70 percent ethanol for 15 min, de-ionized water for 2 min, aged Harris' Hematoxylin for 4 min, de-ionized water for 1 min, 35 percent ethanol for 2 min, 0.5 percent H_2SO_4 in 70 percent ethanol for 7 sec, 70 percent ethanol for 2 min, 95 percent ethanol for 2 min, two changes of absolute acetone for 3 min, two changes of absolute xylene for 3 min each. Sections then were mounted in Permount on glass slides.

The mounted sections were examined independently by the author and two observers who had not previously examined cementum annuli in coyote teeth. Among 74 slides ranging from zero to five in number of annuli estimated, the three observers were in agreement concerning the number of annuli present for 50 percent of the animals. They agreed within one

annulus for 82 percent and within two annuli for 91 percent of the animals. Figure 1 illustrates an easily interpreted section with one annulus, while Figure 2 could be interpreted as having either four or five annuli.

To examine the possibility that the slide preparations were not acceptable, 20 canines were sent to a commercial sectioning service for preparation (10 to be sectioned transversely, 10 sagittally). The commercial service (Montana Microscopic, Box 38, Milltown, Montana 59851) provided excellent slide preparations; however, annuli were difficult to interpret. These slides were examined by the same three observers with only 33 percent complete agreement indicating that the cementum-section-preparation technique used in this study was comparable, in terms of precision, to a professional cementum-section-preparation technique. Estimates from the three independent observers were averaged to the nearest whole number of annuli to provide the most feasible estimate of the number of annuli present in the Oklahoma coyote slides. Assuming a May birth date, each coyote was assigned an age, in months, based on its capture date and number of annuli (averaged across three observers) in the dental cementum.

Weights of right and left eye lenses were combined and examined as a possible criterion of age. The weight of the lens was first examined by Lord (1959) as an age criterion for cottontail rabbits (Sylvilagus floridanus). Since then, a large number of studies have examined the weight of the eye lens for possible correlation with age, but few studies have dealt with lens weight in carnivorous mammals. Lord (1961) further investigated the relationship between lens weight and age in the grey fox (Urocyon cinereoargenteus); however, he did not offer

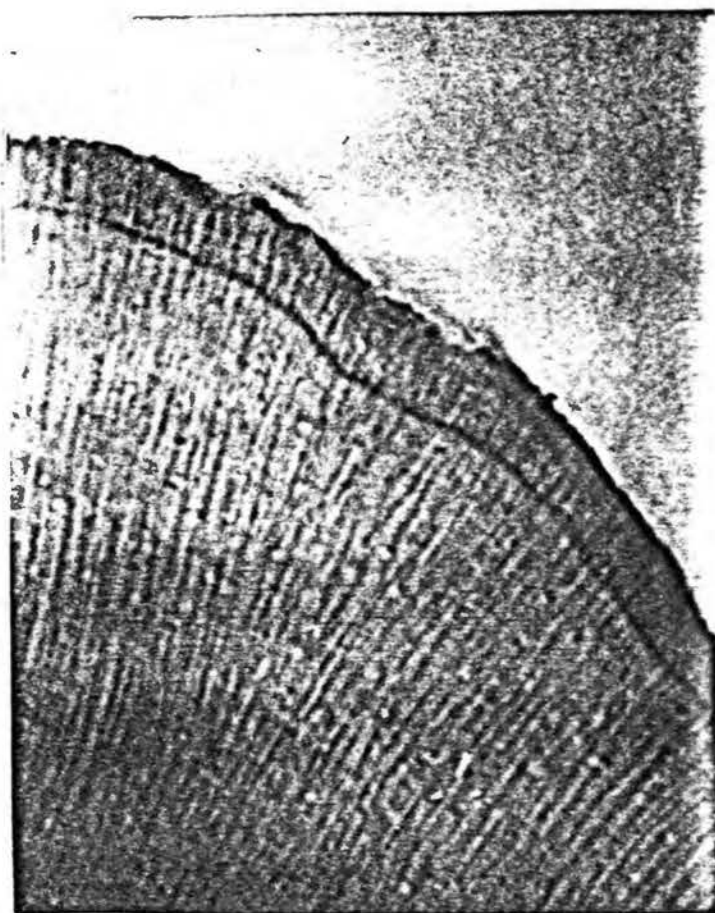


Figure 1. A Cementum Section Showing One
Clearly Definable Annulus



Figure 2. A Cementum Section Which Could Be Interpreted as Having Either Four or Five Annuli

statistical treatment of data adequate for judging the usefulness of the lens as an indicator of age. Bauer et al. (1964) presented data for 164 fur seals (Callorhinus ursinus) via a logarithmically transformed linear regression. They concluded that because 95 percent confidence limits were approximately ± 30 months for animals (male or female) 84 months of age, that lens weight could have little application as a criterion of age in fur seals. Johnston and Beauregard (1969) attempted to use lens weight for aging red foxes (Vulpes vulpes), but great variation in handling procedures prior to fixing led them to abandon the idea.

In view of the paucity of data for carnivorous mammals, the relationship between age, as determined from cementum annuli, and dry weight of the combined lenses was examined for coyotes. After thawing, lenses were removed, cleaned and dried for 120 min in a microwave oven, and weighed to the nearest 0.1 mg on a Mettler analytical balance.

Elder (1951) and Lechleitner (1954) examined the weights of bacula from 234 and 170, respectively, known-age male mink (Mustela vison). In both studies, simple statistical analyses (mean ± 1 standard deviation = 95 percent confidence limits) indicated that the weight of the baculum was an inadequate criterion for distinguishing adults from juveniles in 95 percent of the cases but was adequate in 60 percent of the cases (no overlap ± 2 standard deviations). Friley (1949) reported that male river otters (Lutra canadensis) may be placed into four age classes on the basis of an index assigned by taking the sum of the products of baculum length by weight and length by volume. However, he clearly indicated that his sample of 83 otter bacula was of unknown age by stating that 90 percent were obtained from trappers with the only

data being the dates of capture of 49 animals. For 18 known-age grey foxes, Petrides (1950) found that juvenile foxes could not be distinguished from adults in 95 percent of the cases (analysis similar to that of Lechleitner, 1954).

Bacula were removed and fixed in 10 percent neutral buffered formalin in the present study. Bacula were trimmed of adhering tissue, air dried at room temperature for 30 days, and then weighed to the nearest 0.01 g on a Mettler analytical balance. Baculum length was measured to the nearest 1.0 mm using a Vernier caliper and volume was measured to the nearest 0.5 cc by using displacement of water in a graduated cylinder.

Verzar (1956) demonstrated that there exists a relationship between thermal contraction of weighted (0.1 to 0.2 g) tail tendons of inbred white rats and their ages. While the exact biochemical nature of thermal contractions is not yet completely understood, Banga et al. (1954) suggest that contraction may be due to liberation of dicarboxylic amino acids under heating. According to Bear (1953), hydrogen bonds that maintain the helical structure of collagen (the main protein composing tendons) are destroyed during heating. To my knowledge, an evaluation of thermal contraction of tendons as an age criterion has not been attempted for any wild species.

Individual coyote tail tendons were dissected from the tendon packets. Tendon diameter was standardized with an optical micrometer to 0.60 ± 0.05 mm, weight to 15.0 ± 2.0 mg with a Mettler analytical balance, and length to 100 ± 0.5 mm with a stainless steel rule. Three individual tendons were immersed in Ringer's solution at 62 C for 7

minutes, and the amount of contraction of three tendon segments was recorded in millimeters for each of 21 coyotes.

CHAPTER III

RESULTS

Data derived from each of the three potential age criteria were analysed by simple linear regression. Regression analysis was chosen because it can provide reasonably detailed analysis of the relationship between two changing variables (provided that they are changing linearly). Preliminary plots of datum points for each of the three tested criteria indicated, and later statistical tests confirmed, that the relationship was linear in all cases ($P < 0.05$). The 95 percent level is used throughout this study for both statistical tests and confidence limits.

Draper and Smith (1966) suggest that a test statistic be generated by analysis of variance to determine when a relationship is linear. The test is conducted as a standard F-test. The F-statistic is generated by dividing the regression mean square by the residual mean square. A generated F-statistic of lesser magnitude than the corresponding tabulated F would lead to the rejection of the hypothesis that the data are nonlinear. Under this condition, a linear model may be accepted for adequacy of fit.

In order to validly compare the three age criteria, the confidence limits for each must be calculated in the same manner, because different confidence interval formulae deliver slightly different results. All 95 percent confidence limits in this study were calculated from a

modification of the formula (6.14.1) suggested by Snedecor and Cochran (1967):

$$(X_U, X_L) = \tilde{X} \pm \frac{t S_{y.x}}{B1} \sqrt{1 + \frac{1}{n} + \frac{\tilde{X}^2}{\sum X^2}}$$

where:

\tilde{X} = Predicted value of X

t = Student's t at .05 with n-2 df

B1 = Slope of regression line

$S_{y.x}^2$ = Error variance

Bo = intercept of regression line

$1/\tilde{X} = -Bo/B1 + \log_e Y/B1$

The model $Y = A + BX$ proved inadequate for describing the relationship between coyote eye lens weight and age, suggesting the need for more detailed modeling. Seven models were evaluated by Ericksen et al. (1970) for suitability in describing the relationship between weight and age of mule deer eye lens. The model $\log_e Y = A + B (1/X)$ was found to have the highest correlation of any of the seven models tested for combined male and female mule deer, and a test for "goodness-of-fit" indicated an adequate model.

The model suggested by Ericksen et al. (1970) was evaluated for the relationship between coyote eye lens weight and age. Figure 3 shows the line: $\log_e Y = 0.80652 + 3.146569 (1/X)$ fitted through the datum points. There was no significant departure from linearity in the model (indicating an adequate model), and significant deviation from the horizontal was present in the prediction of Y given X. The analysis of variance is shown in Table I. Selected 95 percent confidence limits about mean and individual predictions are listed in Table II.

Both mean and individual limits are presented for comparative

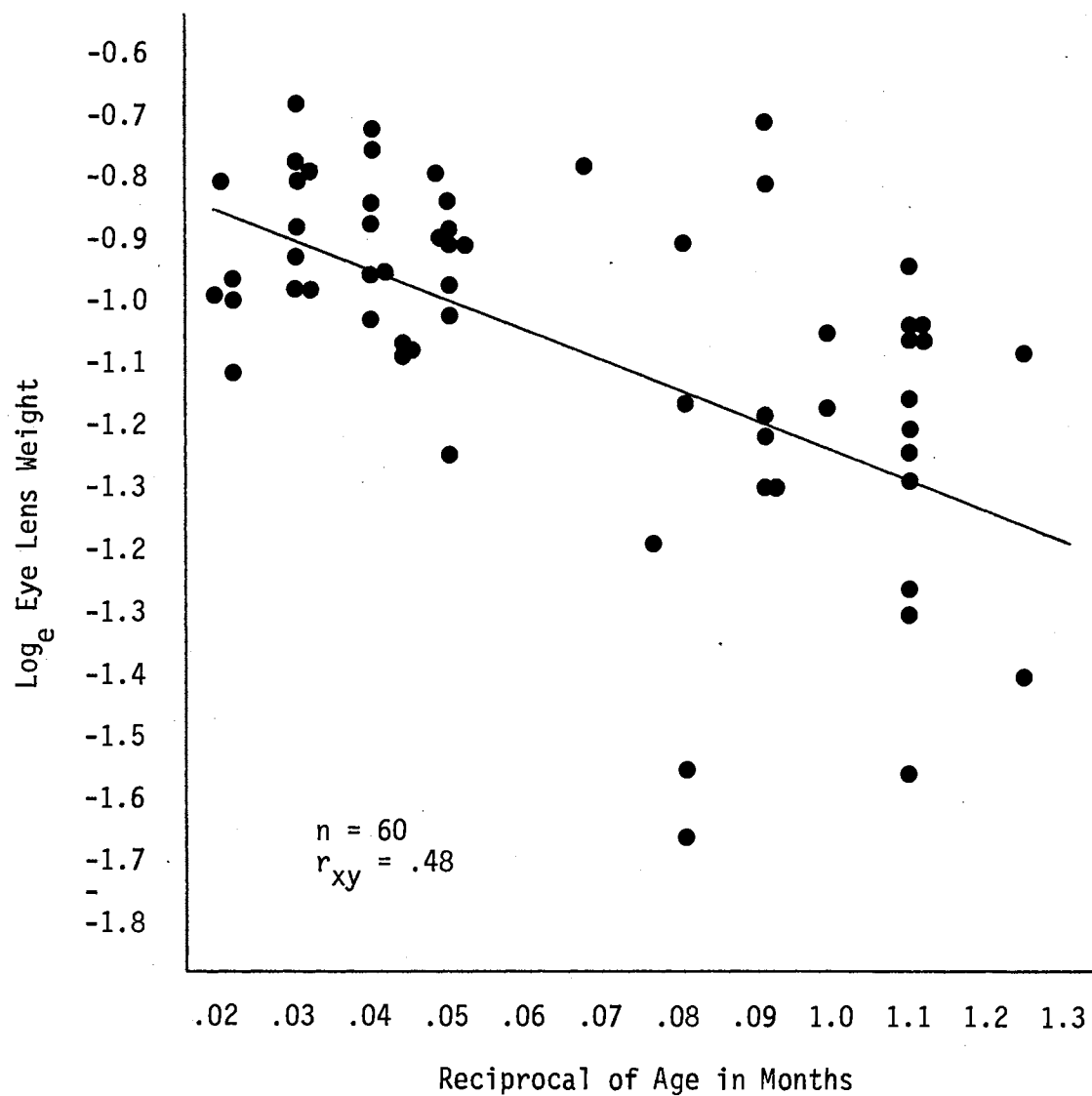


Figure 3. The Transformed Regression of Coyote Eye Lens Weight on Age

TABLE I
THE ANALYSIS OF VARIANCE TABLE FOR THE
REGRESSION OF COYOTE EYE LENS
WEIGHT ON AGE

Source	df	SS	MS	F
Corrected Total	59	2.827482		18.5906
Due to Regression	1	0.686311	0.686311	0.1341
Lack of Fit	12	0.078071	0.006506	
True Error	46	2.063100	0.048500	
Residual	58	2.141171	0.036917	

TABLE II
THE 95 PERCENT CONFIDENCE LIMITS ABOUT THE AGES
OF INDIVIDUAL COYOTES PREDICTED FROM
REGRESSION OF COYOTE EYE LENS
WEIGHT ON AGE

$\log_e Y$	Predicted Age (months)	Individual Lower Limit	Individual Upper Limit	Mean Lower Limit	Mean Upper Limit
-1.8	3.2	1.4	5.0	1.5	4.9
-1.6	4.0	1.8	6.2	1.8	6.2
-1.4	5.3	2.4	8.2	2.4	8.2
-1.2	8.0	3.6	12.4	3.6	12.4
-1.0	16.3	7.4	25.2	7.4	25.2
-0.8	undefined				

purposes, because failure to distinguish between the two can result in an erroneous evaluation of an age criterion. Confidence limits for mean predictions are characteristically much narrower than are confidence limits for individual predictions. The presentation of confidence limits for mean predictions labeled simply as confidence limits about predictions could be misleading. Differences between mean and individual predictions obtained from coyote eye lens weights are unusually small. The slight difference is due to a small $(0.341160) \sum X^2$ in the prediction formula resulting from modeling transformation.

Although significance of regression at the 95 percent level was present for eye lens weight on age, the correlation ($r_{xy} = 0.48$) was too weak to permit confidence limits of ± 6 months. Hence, coyotes cannot be placed in a single-year age class with 95 percent accuracy on the basis of eye lens weight.

The relationship between coyote age and the weight of the baculum was examined for 27 coyotes using simple linear regression. A regression line (baculum weight Y on age X) was fitted to the data by the method of least squares yielding the model: $Y = 0.03923X + 0.5452$. Tests for model adequacy and significance of regression were performed from the analysis of variance (Figure 4; Table III); the model proved adequate, and significance of regression was present. Ninety-five percent confidence limits for mean predictions here are typically narrower (Table IV) than the limits for individual age predictions; however, neither mean nor individual limits are ± 6 months or less. Therefore, the weight of the baculum is not an acceptable criterion for 95 percent accurate predictions of individual ages even though it provided the strongest correlation ($r_{xy} = 0.73$) with age of the three

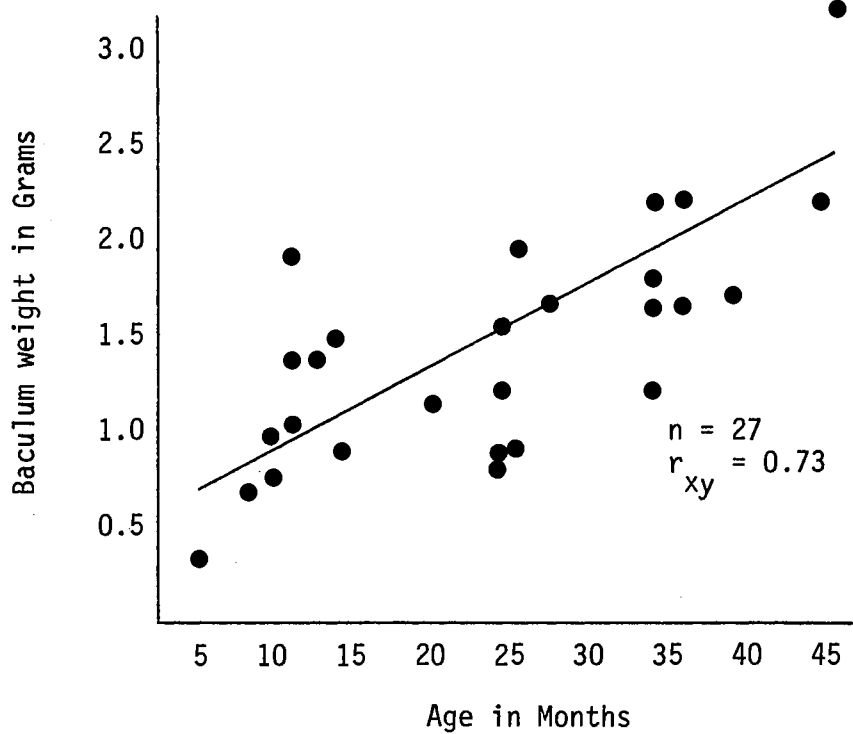


Figure 4. The Regression of Coyote Baculum Weight on Age

TABLE III
THE ANALYSIS OF VARIANCE TABLE FOR THE
REGRESSION OF COYOTE BACULUM
WEIGHT ON AGE

Source	df	SS	MS	F
Corrected Total	26	10.0110		
Due to Regression	1	5.3568	5.3568	28.7691
Lack of Fit	14	2.3980	0.1713	0.8352
True Error	11	2.2562	0.2051	
Residual	25	4.6542	0.1862	

TABLE IV
THE 95 PERCENT CONFIDENCE LIMITS ABOUT THE AGES
OF INDIVIDUAL COYOTES PREDICTED FROM THE
REGRESSION OF BACULUM WEIGHT
ON AGE

Level of Y (gm)	Predicted Age (months)	Individual Lower Limit	Individual Upper Limit	Mean Lower Limit	Mean Upper Limit
.5	undefined				
1.0	11.5	-11.7	34.7	6.7	16.3
1.5	24.3	0.8	47.8	18.2	30.4
2.0	37.1	13.1	61.1	29.3	44.9
2.5	49.8	25.2	74.4	40.1	59.5

criteria examined.

The regression of the length of thermal contraction of coyote tendons on age was significant. The model $Y = 0.33145X + 53.471$ (Figure 5) was proven adequate by the test described earlier. Analysis of variance is tabulated in Table V, and 95 percent confidence limits for mean and individual predictions are summarized in Table VI. Of the three criteria examined, length of tendon contraction provided the weakest correlation ($r_{xy} = 0.40$) with age; correspondingly, confidence limits were broadest and far in excess of ± 6 months.

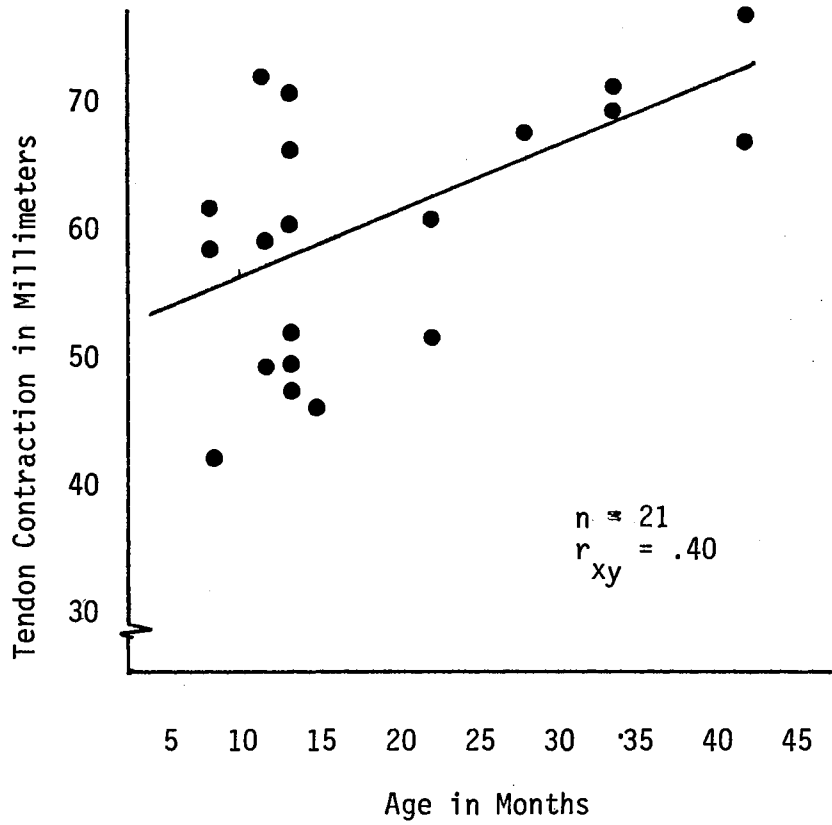


Figure 5. The Regression of Coyote Tail Tendon Contraction on Age

TABLE V
THE ANALYSIS OF VARIANCE TABLE FOR THE
REGRESSION OF COYOTE TENDON
CONTRACTILITY ON AGE

Source	df	SS	MS	F
Corrected Total	62	6621.9300		
Due to Regression	1	838.4241	838.4241	8.8430
Lack of Fit	16	1769.3659	110.5853	1.2397
True Error	45	4014.1400	89.2031	
Residual	61	5783.5059	94.8115	

TABLE VI
THE 95 PERCENT CONFIDENCE LIMITS ABOUT THE AGES
OF INDIVIDUAL COYOTES PREDICTED FROM THE
REGRESSION OF COYOTE TENDON
CONTRACTILITY ON AGE

Level of Y (mm)	Predicted Age (months)	Individual Lower Limit	Individual Upper Limit	Mean Lower Limit	Mean Upper Limit
50.0	undefined				
55.0	4.6	-54.7	63.9	-3.0	12.2
60.0	19.7	-39.9	79.3	9.6	29.8
65.0	34.8	-25.6	95.2	20.6	49.0
70.0	49.9	-11.8	111.6	31.1	68.7

CHAPTER IV

DISCUSSION

Population research and management needs, especially the latter, have necessitated the development of many aging criteria. Linhart and Knowlton (1967) indicated that the age of individual coyotes can be determined reliably by counting cementum layers in the canine teeth. Their study was based on 30 known-age coyotes. They did not quantify the accuracy and precision of their technique.

Monson et al. (1973) reported the accuracy of counting annuli in sectioned canine and premolar teeth of 50 known-age red foxes. They reported that three independent investigators had difficulty "reading" 35 canine tooth sections, and accuracy was reported as 45.7 percent based on the proportion of samples aged correctly in each age class by at least two of the three observers.

By examining premolar teeth, all three observers correctly determined the age of 27 of the 50 specimens, and two of the three observers agreed on the age of 18 others. This was interpreted as an accuracy of 90 percent because 45 specimens were aged correctly by at least two of the three observers. The 45.7 and the 90 percent accuracy stated for canine and premolar teeth respectively, is incorrect. It is invalid to discard the results of one of the three observers, the result being a confused measure of accuracy without measure of precision. For example, to be correct, Monson et al. (1973) should have

reported 54 percent precision with 100 percent accuracy based upon interpretation of premolar sections.

The hesitancy of investigators to report precision of studies on cementum annuli suggests the possibility that it may be lower than implied. In this study, all three investigators had difficulty "reading" certain of the cementum sections. Other sections were clearly definable suggesting considerable individual variability in the aspects of dental cementum responsible for visibility of annuli. With only 50 percent complete agreement between the three observers (50 percent precision), the known ages in the present study are certainly suspect; although it is impossible to predict whether evaluation of the three criteria used in this study was adversely affected by inaccurate assessments of age.

Inconsistent handling of study animals in the field (time before freezing) may possibly have contributed to the variability of the three criteria. Coyote tendons and eye lenses are probably more sensitive than bacula to inconsistent handling. This may be reflected in the observation that of the three criteria tested, baculum weights gave the highest correlation with age.

Further confounding of results was avoided by utilizing consistent statistical procedures. The generally accepted 95 percent confidence level and the same confidence interval calculation formula were used for each of the criteria. The narrowest confidence limits, provided by the baculum weight criterion, were approximately ± 22 months. The absence of studies on coyote cementum annuli that provide confidence limits prevents comparison at this time.

In terms of comparative simplicity, the three criteria examined

in this study appear to be superior to cementum annuli determinations. Cementum annuli determinations are time consuming, expensive and apparently require experience for proper interpretation or "reading" of annuli. The three study criteria involve only simple dissection and standard laboratory measurements.

It is apparent that a comparison between studies incorporating cementum annuli counts from known-age coyotes, presenting valid statements of precision and accuracy, and studies on known-age animals presenting statements of precision and accuracy associated with other age criteria are needed. At present, the relative merits of cementum annuli and other age criteria evaluated in this study appear to be equal.

CHAPTER V

SUMMARY

Current histological procedures for obtaining annuli counts in tooth cementum are undesirable because of required costs, equipment and expenditure of time. In an attempt to obtain a simpler aging method, three criteria were examined (baculum weight, eye lens weight and thermal contraction of tendons) in this study to determine whether coyotes could be placed into a single-year age class. Coyotes were collected throughout Oklahoma and the cementum annuli in the canines were counted. The coyotes' ages, as determined by the cementum annuli method, were used as the standard against which the other techniques were compared. Analyses were confined to simple linear regression for all of the criteria evaluated. Significance ($P < 0.05$) of regression and model adequacy were demonstrated for each of the three potential criteria. Correlation coefficients of 0.73, 0.48 and 0.40 were calculated for baculum weight, eye lens weight and tendon contractility, respectively. Correlations were not sufficiently strong to provide 95 percent confidence limits of ± 6 months or less for individual age predictions. It was concluded that none of the three potential criteria could be used with 95 percent confidence to assign individual coyotes to a single-year class. Compared with cementum annuli aging methods,

the three criteria are simpler to use; and in the absence of quantitative statements of precision and accuracy associated with cementum annuli, appear to have equal merit.

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